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Correlation of del13q, del11q and Trisomy 12 with Laboratory and Clinical Features of Chronic Lymphocytic Leukemia in Iranian Patients

H Teimori[1], MT Akbari[1]*, GR Toogeh[2], M Khaleghian[2]

[1]Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, [2]Emam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background: There is a strong association between chromosomal abnormalities and laboratory features and clinical course of the B-cell chronic lymphocytic leukemia (B-CLL). The aim of this study was to investigate the frequency and correlation of cytogenetic aberrations with laboratory and clinical features of the disease.

Methods: Clinical and laboratory features of 65 CLL patients were collected from their hospital profiles and their blood and/or bone marrow were examined by conventional cytogenetics and interphase FISH methods.

Results: Conventional cytogenetic methods identified 27.7% chromosomal abnormalities in 65 patients. I-FISH analysis for del13q, del11q and trisomy 12 revealed abnormality in 75.4% of patients. The results showed that I-FISH improved the detection rate of chromosomal abnormalities and it enhanced detection. Statistical analysis was performed on sex, age, family history, Rai stage and CD markers on trisomy 12, del 11q and del 13q subgroups. There was a high frequency of Ray stages I and II within del13q subgroup, Rai stages III and IV within del11q subgroup and Rai stage II within trisomy 12 subgroup. Mean of CD38 in patients with del 11q was significantly higher than mean of patients with trisomy 12 and del 13q.

Conclusion: High level of CD38 and presence of del11q indicated a poor prognosis and low level of CD38 and presence of del13q was indicative of good prognosis in Iranian B-CLL patients. Trisomy 12 had an intermediate prognostic value.

Keywords: Chronic lymphocytic leukemia; Chromosomal aberrations; Rai stages; CD markers; Iran

Introduction

B-cell Chronic Lymphocytic Leukemia (B-CLL) is the most frequent leukemia in Western countries and has an annual incidence of 20 new cases per 100,000 inhabitants above age of 60 years and comprises 30% of all leukemia patients. The gender ratio varies from 1.5 to 2.1 males to 1 female.[1] It is still a common cancer in Iran.[2] B-CLL is characterized by the relentless accumulation of monoclonal B-cell with the appearance of small mature lymphocytes and with characteristic immunophenotype (CD5, CD19, CD20, and CD23 positive).[3]

Clinical course of the disease is highly variable. Some patients have stable disease with normal surviving without requiring any specific therapy, while others develop a progressive form of the disease despite aggressive treatments.[4] Transplantation of hematopoietic stem cells (HSCT) has become the standard treatment for many patients with Chronic Lymphocytic Leukemia.[5] Patients are asymptomatic at presentation but with progression of the disease, they develop fatigue, autoimmune hemolytic anemia, infection, splenomegaly and lymphadenopathy.[2,6] Because of the variable phenotype of the disease, several research groups have tried to identify prognostic factors to predict the clinical outcome of the disease.[7] Age, sex, clinical staging (Rai or Binet staging), blood lymphocyte count, lymphocyte doubling time, morphological

***Correspondence:** Mohammad Taghi Akbari, MD, Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Jalal-al-Ahmad Expressway, Tehran, Iran. Tel: +98-21-82884517, Fax: +98-21-82884555, e-mail: mtakbari@modares.ac.ir
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cell features, immunophenotype and genetic markers have all been identified as important prognostic factors.[8] Chromosomal abnormalities are the genetic markers that play an important role in diagnosis and prediction of B-CLL. Deletion in 13q14, 11q22, 17p13 and trisomy 12 are the most common chromosomal abnormalities that have been observed in B-CLL. Deletion in 11q22 and 17p13 are associated with poor prognosis and deletion of 13q14 was demonstrated to have a good prognosis.[9] Trisomy 12 is considered to confer a worse prognosis.[10] Conventional cytogenetics and interphase FISH analysis routinely are used to define chromosomal abnormalities in B-CLL. Conventional cytogenetics can detect 40-50 % of abnormalities. The use of cytogenetic analysis has been often hampered by low in vitro mitotic rate of leukemic B-cells and the poor response to mitogens.[11] Interphase FISH is more powerful and sensitive, and it can detect 80% of chromosomal abnormalities.[12]

The aims of this study were first to investigate the incidence of chromosomal abnormalities in B-CLL patients undergoing conventional cytogenetic methods and FISH technique and second to evaluate the correlation between these abnormalities and prognostic factors such as age, sex, blood lymphocyte count, family history, Rai staging and CD markers.

Materials and Methods

Patients were recruited during 20 months between 2006 and 2008 from four major hematology/oncology hospitals in Tehran, Capital of Iran. The patients were either new subjects or they were already identified as B-CLL cases and were not taking any therapeutic treatment six months prior to sampling. Their diagnostic inclusion criteria were based on the National Cancer Institute Working Group (NCI-WG) guidelines for diagnosis of B-CLL.[13] Seventy patients were enrolled. Immunophenotypic data and blood parameters were collected from patient's hospital files; and questions on age, family history, onset of disease and treatment measures were also provided. Patients having more than either one first degree relative or two second degree relatives affected by any type of cancer in their family were considered as positive family history.

Blood samples and /or bone marrow aspiration were taken in heparinazed collection tubes and were transferred immediately to laboratory for experimental work. The study was approved in the Ethical Committee of the School of Medical Sciences of Tarbiat Modares University. Each patient was also informed of the enrollment procedure for taking part in the study and signed the written consent.

Peripheral blood and bone marrow samples were differently pretreated for culturing. Bone marrow from 22 and peripheral blood from 36 patients were provided. Both specimens were also collected from 7 patients. Peripheral blood was first washed with minimal culture medium (RPMI-1960 with no further supplementation) three times and then white blood cells (WBC) were counted by Neubauer hemocytometer. Bone marrow specimens only were counted by Neubauer hemocytometer. 10^6cells/ml was cultured in 5 ml completed culture medium [RPMI-1960 medium (Gibco, USA), 10% fetal bovine serum (Gibco, USA), 1% antibiotics and 1% L-glutamine (Gibco,USA)]. Five cultures were set up for each patient whose conditions were as follows: overnight (ONC), 24 hours and 72 hours without mitogen stimulation and 24 hours and 72 hours with phorbol myristate acetate (50 ng/mL) (Biomol, Germany). Harvesting and slide preparation took place according to standard cytogenetic methods (Hypotonic treatment, and methanol-acetic acid (Merk, Germany; 3:1 ratio of fixation). G-banding technique was used. Up to 20 metaphases were analyzed for each patient utilizing Applied Imaging Powergene Intelligent Karyotyping software (Applied Imaging, USA) according to International System for Human Cytogenetic Nomenclature.[14]

The FISH analysis was performed on slides taken from the 24-hours culture without stimulation. Dual-color probes were used to evaluate del 13q14 and del 11q22 (Cytocell, UK) but trisomy 12 was analyzed by centromeric probe purchased from Kreatech Diagnostics (Netherlands). FISH assay was conducted according to the manufacture's instructions. For each probe per patient, two hundred nuclei were analyzed by Zeiss Axioplan 25 position fluorescence microscope (Zeiss, Germany). PowerGene software (MacProbe Version 4.3, USA) was used to capture images. The cut off point for positive values (i.e. Mean of normal control +3 standard deviations) established in bone marrow and peripheral blood from 6 controls with CML, were 8.7 for del 13q14, 7.71 for del 11q22 and 7.2 for trisomy 12 probes.

SPSS software program (SPSS for Windows, version 15, Chicago, IL, USA) was used for statistical analysis. Correlations between Rai staging and CD markers were measured by means of Spearman correlation coefficient. Comparison of numerical variables

such as CD markers, WBC and lymphocyte count between three cytogenetic subgroups were performed using ANOVA test. Other variables such as Rai staging and family history between cytogenetic subgroups were analyzed using Chi Square, Kruskall Wallis and Fisher's Exact tests respectively. The association of untreated and post-treatment categories with chromosomal abnormalities was analyzed using Fisher's Exact test. A p value less than 0.05 was considered significant.

Results

The culture of five cases failed and 65 specimens were successfully analyzed. Patients' clinical characteristics were summarized in Table 1. Forty six cases were male and 19 were female. Gender ratio was 2.8 males to 1 female. Patients were between 42 to 81 years old. The mean age of the patients was 62.3±1.3 years old. They could be classified into three subgroups. Twenty four patients presented with B-CLL diagnosis in the course of this study (new cases). Following the medical diagnosis, they were recruited to this study and were subjected to clinical and laboratory examination. Forty one cases suffered from the disease from 13 to 300 months. Nevertheless, patients of this category could be further subdivided into two groups in terms of clinical outcome: 27 patients requiring treatment (including chemotherapy) and 14 having no need to treatment. The first group was designated as post-treatment patients and was off treatment in the six months prior to entry to this study, whereas the second group did not require stringent treatment and just a routine blood test as check up was carried out.

Analysis of bone marrow and peripheral blood of seven patients showed the same results, i.e. similar abnormalities were observed by karyotyping and FISH methods. Number of the bone marrow specimens in Table 1 is actually the sum of cases with bone marrow and those who provided both bone marrow and peripheral blood. Distribution of Rai staging and family history is also available in Table 1.

Conventional cytogenetic methods revealed chromosomal abnormalities in 18 cases (27.7%). Deletion 13q (five cases), deletion 11q (four cases) and trisomy 12 (five cases) were the most common abnormalities in all cases. Monosomy 21 and monosomy 17 were also identified in three and one patients respectively. Complex abnormalities were observed in 12 cases but, only in one metaphase in each case, however they were not included.

Interphase FISH for del 13q was performed on 53 patients; out of which 21 cases (21/53 or 40%) demonstrated the microdeletion; and 64 patients were analyzed for del 11q; out of which 13 cases (13/64 or 20.3%) turned out to have the microdeletion. Among all cases analyzed by FISH method for trisomy 12, fifteen cases (15/62 or 24%) revealed chromosomal abnormalities. In total, about 75.4% of all cases (49/65) showed the abnormalities by FISH technique. Clinical characteristics of these three subgroups and undetected group have been provided in Table 1.

The range of abnormalities was between 23% and 60% in different patient's specimens. In comparison to cut off values, this range verified the abnormalities in the patients properly.

Fourteen patients who were detected by conventional cytogenetic techniques as trisomy 12, del 11q and del 13q were confirmed by FISH method. The patients with monosomy 21 and 17 (4 cases of 65 patients or 6.1%) were just identified by conventional cytogenetic methods. Therefore FISH and conventional cytogenetic methods together identified 81.5%

Table 1: Clinical and laboratory characteristics of Iranian B-CLL patients

Patient	No.	Sex		Age				Tissue			Rai stage				Fam. His.	
		M	F					BM	PB	O	I	II	III	IV	Pos	Neg
				<50	50-60	60-70	>70									
All	65	46	19	11	17	23	14	29	36	7	11	28	14	5	21	44
Del 13q	21	15	6	3	5	8	5	11	10	4	5	12	0	0	5	16
Del 11q	13	9	4	3	5	4	1	4	9	0	2	2	6	3	5	8
Trisomy 12	15	11	4	3	3	5	4	6	9	1	1	8	4	1	5	10
Undetected	16	11	5	2	4	6	4	6	10	2	3	6	4	1	6	10

Abbreviations: No., number; Fam. His., family history; M, male; F, female; <50, less than 50 years; 50-60, between 50 and 60 years; 60-70, between 60 and 70 years; >70, more than 70 years; BM, bone marrow; PB, peripheral blood; Pos, positive; Neg, negative

(53/65) of patients as having chromosomal abnormalities. A brief overview of cytogenetics and FISH results were illustrated in Figure 1.

Correlation of Rai staging with CD38 was significant (p=0.05), but it was not significant with CD5, CD19, CD20, CD23, WBC, PLT, age and family history. Secondly we selected patients with trisomy12, 13q and 11q deletion and analyzed those three subgroups with other variables. Rai staging in three subgroups had a significant distribution (Chi square 11.4 at p=0.01 level). Trisomy 12 was mostly distributed in Rai stage II, del 13q was mainly seen in low level Rai stages (I and II), and 11q deletion patients tended to cluster with high level of Rai stages (III and IV). As far as the association between family history and three subgroups was concerned, analysis by Fisher's Exact test did not show any significance (p=0.302 for 1-side and p=0.417 for 2-side Exact significance). Age, CD5, CD19, CD20, CD23, did not have significance at the 0.05 in the two subgroups; just CD38 was significant (p=0.002). It means that patients with del 11q, trisomy 12 and del 13q had high, moderate and low value of CD38 respectively.

Fisher's Exact test was significant (p=0.026 for 1-side and p=0.031 for 2-side Exact significance). It showed that post treatment subgroup correlated with trisomy 12 and del 11q abnormalities, whereas untreated subgroup correlated with del 13q.

FISH analysis revealed 75.4% chromosomal abnormality in total, comprising 40% for del 13q, 20.3% for del 11q and 24% for trisomy 12. The results of present study and the other published studies have been summarized in Table 2.

Discussion

Although CLL is the most prevalent type of leukemia, its annual incidence of new cases is low and as a result the number of new cases is generally low.[2] So the number of new cases comprised slightly over a third of the present study. However some female patients refrained from taking part in the study, as a result, the gender ratio shifted towards more males. There were two reasons why bone marrow specimen was less than peripheral blood samples. First, a great number of recruited patients were those who had already been diagnosed as B-CLL and the physicians did not feel necessary to perform another bone marrow aspiration, so patients with bone marrow specimen reduced. Second, following careful analysis of seven patients from whom, we had both bone marrow and peripheral blood specimens, which demonstrated no differences in cytogenetic outcome between the two tissues,

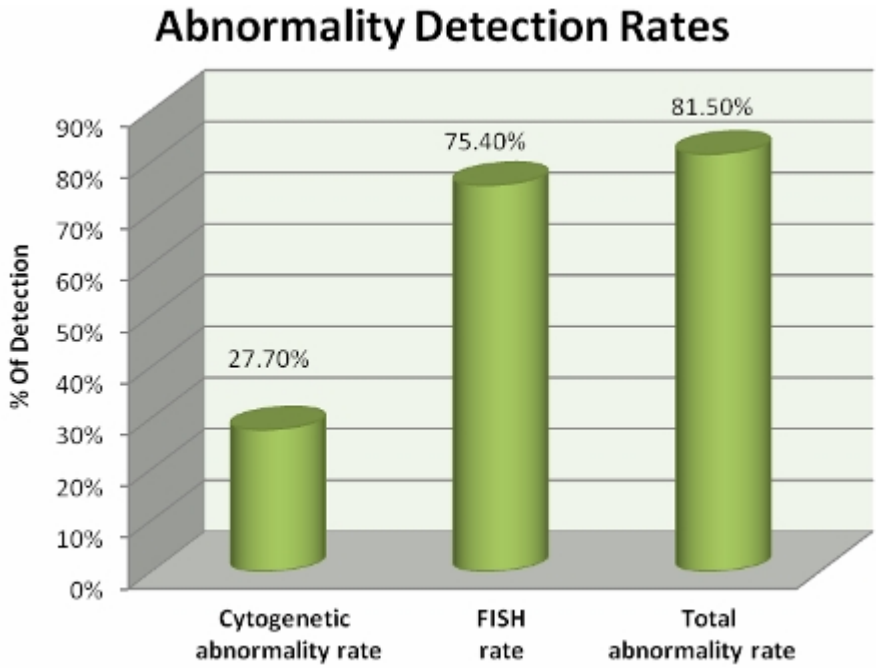

Fig. 1: Bar chart of detection rate of conventional cytogenetic methods, FISH technique and total rate.

Table 2: Detection rates of three cytogenetic subgroups analyzed by FISH in different studies

Reference	Country	Del 13q (%)	Del 11q (%)	Trisomy 12 (%)	FISH Ab. (%)	No.
Amiel *et al.*	Israel[1]	-	5.5	18.5	-	54
Glassman *et al.*	USA[5]	40	23	11	64	100
Aoun *et al.*	USA[9]	46	7	21	72	91
Chang *et al.*	Korea[10]	69	14	19	63	16
Sindelarova *et al.*	Czech[14]	54	12	16	70	217
Ripolles *et al.*[13]	Spain[15]	42.1	17.5	19.2	68.4	65
Chena *et al.*	Argentina[16]	63.2	-	21.9	80.7	57
Dewald *et al.*	USA[17]	64	15	25	77	113
Quijano *et al.*	Spain[18]	35	9	23	62	180
Dohner *et al.*	Germany[19]	55	18	16	82	325
Dorak *et al.*	Turkey[20]	32.9	5.1	15.2	50.6	79
Present study	Iran	40	20.3	24	75.4	65

Abbreviations: Ab., Abnormality; No., number of cases.

patients refusing bone marrow aspiration were enrolled in the study and this increased the number of peripheral blood specimens.

The weak mitogen stimulation of malignant cells in B-CLL and the fact that unlike other forms of leukemia, their cells are in G_0 phase of cell cycle led to a decrease of metaphase in cytogenetic analysis. Moreover most of their chromosomes were short and small and less liable to good banding. These factors made it very difficult to identify structural abnormalities such as translocation, inversion and microdeletion. Almost exclusively numerical gain or loss and very big deletions are recognizable. For these reasons, we could just identify 27.7% of chromosomal abnormalities by conventional cytogenetic methods.

FISH analysis revealed 75.4% chromosomal abnormality in total, comprising 40% for del 13q, 20.3% for del 11q and 24% for trisomy 12. The results of present study and the other published studies have been summarized in Table 2. As it is evident, each of these chromosomal abnormalities have a wide range between different population: 32%-69% for del 13q, 5.1%-23% for del 11q and 11%-25% for trisomy 12. Nevertheless, in all studied groups the order of abnormality with regard to prevalence is similar: the most common being del 13q, followed by trisomy 12 and del 11q. As far as detection rate for FISH analysis was concerned, variation between the studied groups also existed, and our study result was within this range: 50.6%-82%. Comparison of these data indicated clearly that these findings are similar. It could be concluded that the type and the rate of chromosomal aberrations in Iranian B-CLL patients were similar to other countries and the underlying genetic mechanisms leading to the disease could not be different. However, identification of such a higher frequency of abnormalities by FISH technique compared to conventional cytogenetic methods illustrated greater sensevity of FISH analysis as against conventional karyotyping method. Therefore, FISH testing helps outstandingly in establishing diagnosis for these patients.

As far as the correlation between immunophenotyping and cytogenetic results is concerned, we identified that amongst the CD markers only CD38 showed significant correlation with Rai staging. The coefficient of correlation of 0.3 between these two variables showed an intermediate relationship. This means that along with the increase in Rai staging, the CD38 also increases. This finding in its own right confirms the role of CD38 as a prognostic factor. Statistical analysis of distribution of Rai stages within the three cytogenetic subgroups of patients illustrated that stages III and IV on the one hand and I and II on the other hand had significant correlation with del 11q and del 13q respectively Considering the fact that stages III and IV are correlated with worsening of the patient's condition, it could be concluded that del 11q indicate poor prognosis and on the other hand del 13q indicates good prognosis. However, occurrence of trisomy 12 in Rai stage II and weak correlation with CD38 suggested an intermediate prognostic value for this abnormality. Significance of the relation between the mean of CD38 in

del 11q patients compared to CD38 magnitude in del 13q patients once again reiterated the importance of CD38 evaluation for the prognosis of the patients.

Comparison of untreated and post-treatment patients indicated that aberrations of del 11q and trisomy 12 were seen more frequently in those requiring treatment, whereas del 13q was seen more in the untreated subgroup. As stringent treatment such as chemotherapy is carried out in advanced and progressive form of the disease, it could be concluded that del 11q and trisomy 12 aberrations were associated with progressive form and conversely, del 13q correlated with stable and indolent form of the disease.

Cytogenetic and immunophenotypic results were similar in familial and sporadic B-CLL patients. Some other studies that examined familial B-CLL cases have also suggested similar finding.[15,16]

In conclusion, utilization of I-FISH technique enhanced diagnosis rate in B-CLL patients and also higher amount of CD38 and or presence of del 11q indicated poor prognosis whereas lower amount of CD38 and presence of del 13q pointed at good prognosis. Trisomy 12 had an intermediate prognostic value in our B-CLL patients.

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Conflict of interest: None declared.

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